method for constructing a chimeric virus comprising capsid CTS region

L3 ANSWER 3 OF 43 MEDLINE
ACCESSION NUMBER: 1999128066 MEDLINE
DOCUMENT NUMBER: 99128066 PubMed ID: 9930876
TITLE: PCR-mediated recombination: a general method applied to construct chimeric infectious molecular clones of plasma-derived HIV-1 RNA.
AUTHOR: Fang G; Weiser B; Visosky A; Moran T; Burger H
CORPORATE SOURCE: Wadsworth Center, New York State Department of Health, Albany 12201, USA.
CONTRACT NUMBER: RO1AI33334 (NIAID)
U01AI35004 (NIAID)
SOURCE: NATURE MEDICINE, (1999 Feb) 5 (2) 239-42.
Journal code: CG5; 9502015. ISSN: 1078-8956.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199902
ENTRY DATE: Entered STN: 19990311
Last Updated on STN: 19990311
Entered Medline: 19990223

AB A PCR-based approach was developed that provides a powerful tool for engineering recombinant molecules without reliance on restriction sites. DNA sequences were first amplified by high-fidelity PCR using Pfu polymerase; they were then used both as 'megaprimers' and templates in subsequent asymmetric long PCR amplifications to form chimeric clones. To demonstrate the technique, we constructed chimeric **full-length HIV-1 clones** derived from reverse-transcribed plasma viral RNA and proviral LTRs. Biologic characterization of these clones showed that most were infectious in tissue culture and sequence analysis demonstrated an error rate of only one base change in 20 kb of DNA sequence. For PCR-mediated recombination, it is necessary to know the sequence of the 3' and 5' overlapping regions of the desired PCR products. This method may be extended to include construction of chimeras between any DNA fragments lacking sequence homology. Such chimeras may be constructed by introducing overlapping sequences to one of the fragments. To ensure that unwanted mutations have not been introduced into the clones constructed by this method, each clone should be sequenced. Our results demonstrate that by using a high-fidelity polymerase and highly controlled PCR conditions, the PCR-introduced error rate can be greatly minimized. This new procedure may be used to construct infectious chimeras of HIV or SIV for studies of vaccines and pathogenesis. Moreover, the method is designed to exchange viral genes at precise boundaries to study individual gene products from different HIV genomes. It can also be used to construct expression vectors for production of specific proteins or delivery vectors for gene transfer and gene therapy. Finally, the technique described here provides a versatile tool to transfer genes or gene fragments from different sources for genetic investigation and engineering.

These references are to integrase chimera — can be used for heterologous content

L11 ANSWER 1 OF 7 MEDLINE
ACCESSION NUMBER: 2001133186 MEDLINE
DOCUMENT NUMBER: 21064579 PubMed ID: 11125167
TITLE: Characterization of chimeric enzymes between caprine arthritis--encephalitis virus, maedi--visna virus and human immunodeficiency virus type 1 **integrases** expressed in Escherichia coli.
AUTHOR: Berger N; Heller A E; Stormann K D; Pfaff E
CORPORATE SOURCE: Federal Research Centre for Virus Diseases of Animals, Institute for Immunology, Paul-Ehrlich-Strasse 28, D-72076 Tubingen, Germany.
SOURCE: JOURNAL OF GENERAL VIROLOGY, (2001 Jan) 82 (Pt 1) 139-48.
Journal code: I9B; 0077340. ISSN: 0022-1317.
PUB. COUNTRY: England: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200103
ENTRY DATE: Entered STN: 20010404
Last Updated on STN: 20010404
Entered PubMed: 20010126
Entered Medline: 20010301

AB In order to investigate the functions of the three putative lentiviral **integrase** (IN) protein domains on viral DNA specificity and target site selection, enzymatically active chimeric enzymes were constructed using the three wild-type IN proteins of caprine arthritis-encephalitis virus (CAEV), maedi-visna virus (MVV) and human immunodeficiency virus type 1 (**HIV-1**). The chimeric enzymes were expressed in Escherichia coli, purified by affinity chromatography and analysed in vitro for IN-specific endonuclease and integration activities on various DNA substrates. Of the 21 purified chimeric IN proteins constructed, 20 showed distinct site-specific cleavage activity with at least one substrate and six were able to catalyse an efficient integration reaction.

Analysis of the chimeric IN proteins revealed that the central domain together with the C terminus determines the activity and substrate specificity of the enzyme. The N terminus appears to have no considerable influence. Furthermore, an efficient integration activity of CAEV wild-type IN was successfully demonstrated after detailed characterization of the reaction conditions that support optimal enzyme activities of CAEV IN. Also, under the same in vitro assay conditions, MVV and **HIV-1** IN proteins exhibited endonuclease and integration activities, an indispensable prerequisite of domain-swapping experiments. Thus, the following report presents a detailed characterization of the activities of CAEV IN in vitro as well as the analysis of functional chimeric lentiviral IN proteins.

L11 ANSWER 2 OF 7 MEDLINE
ACCESSION NUMBER: 2001111668 MEDLINE
DOCUMENT NUMBER: 20583838 PubMed ID: 11152524
TITLE: Nucleocytoplasmic shuttling by human immunodeficiency virus

type 1 Vpr.

AUTHOR: Sherman M P; de Noronha C M; Heusch M I; Greene S; Greene W C

CORPORATE SOURCE: Gladstone Institute of Virology and Immunology, University of California, San Francisco, California 94141-9100, USA.

CONTRACT NUMBER: P30 MH59037 (NIMH)

SOURCE: JOURNAL OF VIROLOGY, (2001 Feb) 75 (3) 1522-32. Journal code: KCV. ISSN: 0022-538X.

PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200102

ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010202

AB Human immunodeficiency virus type 1 (**HIV-1**) is capable of infecting nondividing cells such as macrophages because the viral preintegration complex is able to actively traverse the limiting nuclear pore due to the redundant and possibly overlapping nuclear import signals present in Vpr, matrix, and **integrase**. We have previously recognized the presence of at least two distinct and novel nuclear import signals residing within Vpr that, unlike matrix and **integrase**, bypass the classical importin alpha/beta-dependent signals and do not require energy or a RanGTP gradient. We now report that the carboxy-terminal region of Vpr (amino acids 73 to 96) contains a bipartite nuclear localization signal (NLS) composed of multiple arginine residues. Surprisingly, when the leucine-rich Vpr(1-71) fragment, previously shown to harbor an NLS, or full-length Vpr is fused to the C terminus of a green fluorescent protein-pyruvate kinase (GFP-PK) **chimera**, the resultant protein is almost exclusively detected in the cytoplasm. However, the addition of leptomycin B (LMB), a potent inhibitor of CRM1-dependent nuclear export, produces a shift from a cytoplasmic localization to a nuclear pattern, suggesting that these Vpr fusion proteins shuttle into and out of the nucleus. Studies of nuclear import with GFP-PK-Vpr fusion proteins in the presence of LMB reveals that both of the leucine-rich alpha-helices are required for effective nuclear uptake and thus define a unique NLS. Using a modified heterokaryon analysis, we have localized the Vpr nuclear export signal to the second leucine-rich helix, overlapping a portion of the amino-terminal nuclear import signal. These studies thus define **HIV-1** Vpr as a nucleocytoplasmic shuttling protein.

L11 ANSWER 3 OF 7 MEDLINE

ACCESSION NUMBER: 2000120938 MEDLINE

DOCUMENT NUMBER: 20120938 PubMed ID: 10653920

TITLE: Mapping target site selection for the non-specific nuclease activities of retroviral **integrase**.

AUTHOR: Katzman M; Sudol M; Pufnock J S; Zeto S; Skinner L M

CORPORATE SOURCE: Department of Medicine, Section of Infectious Diseases, Pennsylvania State University College of Medicine, The Milton S. Hershey Medical Center, 500 University Drive, Mail Services H036, Hershey, PA 17033-2390, USA.. mkatzman@psu.edu

SOURCE: VIRUS RESEARCH, (2000 Jan) 66 (1) 87-100.
Journal code: X98; 8410979. ISSN: 0168-1702.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200004
ENTRY DATE: Entered STN: 20000427
Last Updated on STN: 20000427
Entered Medline: 20000418

AB To identify the parts of retroviral **integrase** that interact with its DNA substrates, we compared the patterns of target site usage by chimeric enzymes and protein fragments in assays that reveal **integrase**'s non-specific nuclease activities. The central region of 12 chimeric proteins between the human immunodeficiency virus type 1 and visna virus **integrases** was found to be responsible for selecting non-viral target DNA sites when small alcohols provide the attacking nucleophilic OH group during non-specific alcoholysis assays. Testing deletion derivatives of the **integrase** protein in this assay, which has similarities to the DNA joining reaction that occurs during retroviral integration, defined a smaller central domain that is sufficient for activity. Thus, this core domain likely contains both the host DNA site and the nucleophile site. Surprisingly, the region of **integrase** responsible for selecting non-viral target DNA sites when the viral DNA end is the attacking nucleophile could not similarly be mapped with the standard oligonucleotide joining assay. We therefore tested the proteins in a more sensitive assay that displays preferred sites of viral DNA insertion in a plasmid DNA target. All 12 **chimeras** yielded novel patterns compared with the wild-type enzymes in this assay, although local insertion patterns indicated that the central domain plays an important role in target site selection. Together, these data suggest that other protein regions must be involved when the attacking nucleophilic group is provided by viral DNA. Because specific recognition of viral DNA ends was previously mapped to the central domain, two different regions of **integrase** must interact with retroviral DNA.

L11 ANSWER 4 OF 7 MEDLINE
ACCESSION NUMBER: 1999160758 MEDLINE
DOCUMENT NUMBER: 99160758 PubMed ID: 10049823
TITLE: A novel Vpr peptide interactor fused to **integrase** (IN) restores integration activity to IN-defective **HIV-1** virions.
AUTHOR: Kulkosky J; BouHamdan M; Geist A; Pomerantz R J
CORPORATE SOURCE: Center for Human Virology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania 19107, USA.. kulkosok1@jeflin.tju.edu
CONTRACT NUMBER: AI3655 (NIAID)
AI38666 (NIAID)
SOURCE: VIROLOGY, (1999 Mar 1) 255 (1) 77-85.
Journal code: XEA; 0110674. ISSN: 0042-6822.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199904
ENTRY DATE: Entered STN: 19990426

Last Updated on STN: 19990426
Entered Medline: 19990413

AB A novel approach to complement human immunodeficiency virus type I (**HIV-1**) **integrase** (IN)-defective virions has been identified. The approach involves fusion of a 23-amino-acid stretch to the N-terminus of wild-type IN and coexpression of this **chimera** with the IN-defective proviral template in virus producing cells. The 23-amino-acid peptide represents a Vpr "interactor," referred to as the the WxxF or WF domain, which apparently leads to docking of the domain along with the fusion partner onto **HIV-1** Vpr, thus permitting virion incorporation of the chimeric protein when expressed, in trans, with other viral products. Transfection of the WF-IN expression plasmid along with **HIV-1** viral clones that produce Vpr, but bear an IN mutation, results in the release of a proportion of viral particles that are competent for integration. The extent of complementation was assessed using the MAGI cell assay, where integration of viral DNA results in the eventual appearance of easily visible multinucleated blue syncytia. The efficiency of dWF-IN (double copy of WF domain) complementation is not improved markedly by incorporation of a **HIV-1** protease cleavage site (PR) between the dWF domain and IN (dWF-PR-IN), unlike that observed with Vpr fusions to IN. Furthermore, the ability of Vpr-PR-IN and dWF-PR-IN to complement IN-defective proviral clones, both of which bear an intervening protease cleavage site, appear comparable. Western blotting analyses using virions isolated through sucrose cushions demonstrate clearly the incorporation of the dWF-IN fusion protein into Vpr containing **HIV-1** particles but not in Vpr-deficient virions. Additional Western blotting analyses indicate that all Vpr-IN and dWF-IN **chimeras**, with or without a PR site, are packaged into virions. The efficiency of virion incorporation of Vpr-IN and dWF-IN **chimeras** appears approximately comparable by Western blotting analysis. The ability of dWF-IN to complement IN-defective proviruses with efficiency similar to that of Vpr-PR-IN and dWF-PR-IN indicates that dWF-IN retains the full complement of functions necessary for integration of proviral DNA and is likely due to the benign nature of this small domain at the amino-terminus of IN.

L11 ANSWER 5 OF 7 MEDLINE

ACCESSION NUMBER: 95363982 MEDLINE
DOCUMENT NUMBER: 95363982 PubMed ID: 7637015

TITLE: Mapping domains of retroviral **integrase** responsible for viral DNA specificity and target site selection by analysis of **chimeras** between human immunodeficiency virus type 1 and visna virus **integrases**.

AUTHOR: Katzman M; Sudol M
CORPORATE SOURCE: Department of Medicine, Milton S. Hershey Medical Center, Pennsylvania State University College of Medicine, Hershey 17033, USA.

CONTRACT NUMBER: R29 AI30759 (NIAID)
SOURCE: JOURNAL OF VIROLOGY, (1995 Sep) 69 (9) 5687-96.
Journal code: KCV; 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199509
ENTRY DATE: Entered STN: 19950921
Last Updated on STN: 19950921
Entered Medline: 19950914

AB Human immunodeficiency virus type 1 (**HIV-1**) and visna virus **integrases** were purified from a bacterial expression system and assayed on oligonucleotide substrates derived from each terminus of human immunodeficiency virus type 1 and visna virus linear DNA. Three differences between the proteins were identified, including levels of specific 3'-end processing, patterns of strand transfer, and target site preferences. To map domains of **integrase** (IN) responsible for viral DNA specificity and target site selection, we constructed and purified chimeric proteins in which the N-terminal, central, and C-terminal regions of these lentiviral **integrases** were exchanged. All six chimeric proteins were active for disintegration, demonstrating that the active site in the central region of each **chimera** maintained a functional conformation. Analysis of endonucleolytic processing activity indicated that the N terminus of IN does not contribute to viral DNA specificity; this function must reside in the central region or C terminus of IN. In the viral DNA integration assay, chimeric proteins gave novel patterns of strand transfer products which did not match that of either wild-type IN. Thus, target site selection with a viral DNA terminus as nucleophile could not be mapped to regions of IN defined by these boundaries and may involve interactions between regions. In contrast, when target site preferences were monitored with a new assay in which glycerol stimulates IN-mediated cleavage of nonviral DNA, **chimeras** clearly segregated between the two wild-type patterns. Target site selection for this nonspecific alcoholysis activity mapped to the central region of IN. This report represents the first detailed description of functional **chimeras** between any two retroviral **integrases**.

L11 ANSWER 6 OF 7 BIOTECHNO COPYRIGHT 2001 Elsevier Science B.V.
ACCESSION NUMBER: 1998:28100723 BIOTECHNO
TITLE: Mapping viral DNA specificity to the central region of **integrase** by using functional human immunodeficiency virus type 1/visna virus chimeric proteins
AUTHOR: Katzman M.; Sudol M.
CORPORATE SOURCE: M. Katzman, Department of Medicine, Pennsylvania State Univ. Coll. Med., Milton S. Hershey Medical Center, P.O. Box 850, Hershey, PA 17033-0850, United States. E-mail: mkatzman@med.hmc.psu.edu
SOURCE: Journal of Virology, (1998), 72/3 (1744-1753), 73 reference(s)
CODEN: JOVIAM ISSN: 0022-538X
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1998:28100723 BIOTECHNO
AB We previously described the construction and analysis of the first set of

functional chimeric lentivirus **integrases**, involving exchange of the N- terminal, central, and C-terminal regions of the human immunodeficiency virus type 1 (**HIV-1**) and visna virus **integrase** (IN) proteins. Based on those results, additional **HIV-1**/visna virus chimeric **integrases** were designed and purified. Each of the chimeric enzymes was functional in at least one oligonucleotide-based IN assay. Of a total of 12 chimeric IN proteins, 3 exhibit specific viral DNA processing, 9 catalyze insertion of viral DNA ends, 12 can reverse that reaction, and 11 are active for nonspecific alcoholysis. Functional data obtained with the processing assay indicate that the central region of the protein is responsible for viral DNA specificity. Target site selection for nonspecific alcoholysis again mapped to the central domain of IN, confirming our previous data indicating that this region can position nonviral DNA for nucleophilic attack. However, the chimeric proteins created patterns of viral DNA insertion distinct from that of either wild- type IN, suggesting that interactions between regions of IN influence target site selection for viral DNA integration. The results support a new model for the functional organization of IN in which viral DNA initially binds nonspecifically to the C-terminal portion of IN but the catalytic central region of the enzyme has a prominent role both in specific recognition of viral DNA ends and in positioning the host DNA for viral DNA integration.

L11 ANSWER 7 OF 7 BIOTECHNO COPYRIGHT 2001 Elsevier Science B.V.
ACCESSION NUMBER: 1994:24314306 BIOTECHNO
TITLE: Monoclonal antibodies against HIV type 1 **integrase**: Clues to molecular structure
AUTHOR: Bizub-Bender D.; Kulkosky J.; Skalka A.M.
CORPORATE SOURCE: Fox Chase Cancer Center, Institute for Cancer Research, 7701 Burholme Avenue,Philadelphia, PA 19111, United States.
SOURCE: AIDS Research and Human Retroviruses, (1994), 10/9 (1105-1115)
CODEN: ARHRE7 ISSN: 0889-2229
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1994:24314306 BIOTECHNO
AB Eleven murine hybridoma clones were selected for their ability to produce anti-**HIV-1 integrase** (IN) antibodies. Competition and epitope mapping studies allowed segregation of the monoclonal antibodies (MAbs) into four distinct classes. The five MAbs that comprise the first class showed high affinity for epitopes within an N-terminal domain of 58 amino acids that includes a conserved zinc finger motif. The second class, with two MAbs, showed high affinity for epitopes within 29 amino acids at the C terminus. Another two MAbs, which constitute the third class, displayed moderate affinities for epitopes that mapped to regions within the highly conserved catalytic core referred to as the D,D(35)E domain. One of these MAbs showed significant cross-reactivity with HIV-2 IN and weak, but detectable, cross-reactivity

with RSV IN. The remaining two MAbs, which comprise the fourth class, exhibited fairly low binding affinities and appeared to recognize epitopes in the zinc finger motif domain as well as the C-terminal half of the IN protein. The MAbs can be used for immunoprecipitation and immunoblotting procedures as well as for purification of **HIV-1** IN protein by affinity chromatography. We show that several can also be used to immunostain viral IN sequences in **HIV-1** -infected T cells, presumably as a component of Gag-Pol precursors. Finally, analysis of our mapping and competition data suggests a structure for mature IN in which the C terminus approaches the central core domain, and the N and C termini touch or are proximal to each other.

These MAbs should prove useful for further analyses of the structure and function of IN both in vitro and in vivo.

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TI Complementation of **CMV** subgroup IA strains in replicase-mediated resistant tobacco plants after co-inoculation with different cucumoviruses.

L4 ANSWER 4 OF 276 AGRICOLA
TI Resistance to Cucumber mosaic virus in cowpea and implications for control of cowpea stunt disease.

L4 ANSWER 5 OF 276 AGRICOLA
TI Field survey of Cucumber mosaic virus subgroups I and II in crop plants in Costa Rica.

L4 ANSWER 6 OF 276 AGRICOLA
TI Resistance phenotypes of transgenic tobacco plants expressing different cucumber mosaic virus (**CMV**) coat protein genes.

L4 ANSWER 7 OF 276 AGRICOLA
TI The effect of eukaryotic expression vectors and adjuvants on DNA vaccines in chickens using an avian influenza model.

L4 ANSWER 8 OF 276 AGRICOLA
TI Identification of markers linked to a celery mosaic virus resistance gene in celery.

L4 ANSWER 9 OF 276 AGRICOLA
TI Umbravirus-encoded movement protein induces tubule formation on the surface of protoplasts and binds RNA incompletely and non-cooperatively.

L4 ANSWER 10 OF 276 AGRICOLA
TI The suppressor of transgene RNA silencing encoded by Cucumber mosaic virus interferes with salicylic acid-mediated virus resistance.

=> d 2 bib ab

L4 ANSWER 2 OF 276 AGRICOLA
AN 2002:40302 AGRICOLA
DN IND23274111
TI Limited movement of Cucumber mosaic virus (**CMV**) in yellow passion flower in Brazil.
AU Gioria, R.; Espinha, L.M.; Rezende, J.A.M.; Gaspar, J.O.; Kitajima, E.W.
AV DNAL (464.8 P692)
SO Plant pathology, Apr 2002. Vol. 51, No. 2. p. 127-133
Publisher: Edinburgh : Blackwell Science Ltd.
CODEN: PLPAAD; ISSN: 0032-0862
NTE Includes references
CY England; United Kingdom
DT Article
FS Non-U.S. Imprint other than FAO
LA English

=> d 1 bib ab

L4 ANSWER 1 OF 276 AGRICOLA
AN 2002:40717 AGRICOLA
DN IND23274696
TI Characterization of synergy between Cucumber mosaic virus and potyviruses in cucurbit hosts.
AU Wang, Y.; Gaba, V.; Yang, J.; Palukaitis, P.; Gal-on, A.
AV DNAL (464.8 P56)
SO Phytopathology, Jan 2002. Vol. 92, No. 1. p. 51-58

Publisher: St. Paul, Minn. : American Phytopathological Society, 1911-
CODEN: PHYTAJ; ISSN: 0031-949X
NTE Includes references
CY Minnesota; United States
DT Article
FS U.S. Imprints not USDA, Experiment or Extension
LA English
AB Mixed infections of cucurbits by Cucumber mosaic virus (**CMV**) and potyviruses exhibit a synergistic interaction. Zucchini squash and melon plants coinfected by the potyvirus Zucchini yellow mosaic virus (ZYMV) and either Fny-**CMV** (subgroup IA) or LS-**CMV** (subgroup II) displayed strong synergistic pathological responses, eventually progressing to vascular wilt and plant death. Accumulation of Fny- or LS-**CMV** RNAs in a mixed infection with ZYMV in zucchini squash was slightly higher than infection with **CMV** strains alone. There was an increase in **CMV** (+) strand RNA levels, but no increase in **CMV** (-) RNA3 levels during mixed infection with ZYMV. Moreover, only the level of capsid protein from LS-**CMV** increased in mixed infection. ZYMV accumulated to similar levels in singly and mixed infected zucchini squash and melon plants. Coinfection of squash with the potyvirus Watermelon mosaic virus (WMV) and **CMV** strains increased both the Fny-**CMV** RNA levels and the LS-**CMV** RNA levels. However, **CMV** (-) strand RNA3 levels were increased little or not at all for **CMV** on coinfection with WMV. Infection of **CMV** strains (LS and Fny) containing satellite RNAs (WL47-sat RNA and B5*-sat RNA) reduced the accumulation of the helper virus RNA, except when B5*-sat RNA was mixed with LS-**CMV**. However, mixed infection containing ZYMV and the **CMV** strains with satellites reversed the suppression effect of satellite RNAs on helper virus accumulation and increased satellite RNA accumulation. The synergistic interaction between **CMV** and potyviruses in cucurbits exhibited different features from that documented in tobacco, indicating there are differences in the mechanisms of potyvirus synergistic phenomena.